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PHYTOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT POTENTIAL OF LITCHI SEEDS

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Abstract

Phytochemicals act against free radicals and are formed in human body continuously and damage body cells, causing many diseases like cellular damage, cancer, etc. Plant and their different parts are the main sources of phytochemicals. Litchi (*Litchi chinensis* Sonn.*)* is one of the most popular fruits of which only flesh is consumed by humans and the rest of the parts especially seeds are discarded. This study has been carried out to detect antioxidant potential in various extracts (aqueous and ethanol) of Bombay litchi variety seeds and to discover the contextual connection of phytochemicals as antioxidant activities. The collected seeds were washed, dried in sunlight, and finally ground to fine powder to a uniform particle size for extraction and isolation of phytochemicals. The qualitative and quantitative phytochemical analysis and antioxidant activities were determined using standard methods. Phytochemical screening reveals that most of the phytochemicals were extracted from litchi seed in ethanol. TPC and TFCs of ethanol extract were 19.86 and 20.60 mg/g, respectively. The antioxidant activity was estimated by DPPH assay method from litchi seed extracts, and IC_{50} of the seed phytochemicals and ascorbic acid was observed to be 274 and 240 µg/ml, respectively. On the other hand, free radical scavenging activities of seed phytochemicals were determined with ABTS assay; IC_{50} of seed phytochemicals and ascorbic acid was noted as 31 and 29 µg/ml, respectively. Results suggested that the litchi seed is one of the richest sources of phytochemicals that can be used as a source of bioactive compounds. It is concluded that bioactive litchi seed phytochemicals can be used as herbal medicine defense against aging, oxidative stress, cell damage as well as cancer.

Keywords: Antioxidant, Free radicals, *Litchi chinensis* Sonn, Phytochemicals

Introduction

Phytochemicals are plant-based biologically active natural substances that have the antioxidant and medicinal activity to provide health benefits for humans (Hasler and Blumberg, 1999; Zhao *et al*., 2015). In general, phytochemicals protect plants from environmental stresses, diseases, and contribute to the plant's color and flavor (Koche *et*

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al., 2016). Different parts of a plant such as a leaf, bark, fruits, seeds, etc. produce a large number of bioactive compounds that protect from free radical damage thus known as natural antioxidants and act against aging (Hasler and Blumberg, 1999; Zhao *et al*., 2015). Oxidative stress caused by free radicals is associated with the development of numerous diseases such as cardiovascular, neurodegenerative, autoimmune diseases, cancers, etc. (Valko *et al.*, 2006). Medicinal plants contain various phytochemicals that have high antioxidant activities. Consequently, current research on naturally occurring bioactive compounds especially from plant sources has a great concern for lessening oxidative stress that causes free radicals randomly formed in our body. Natural antioxidants have no side effects. Antioxidants reduce oxidative damage by delaying or inhibiting the production of reactive oxygen species or reactive nitrogen species and ultimately reduce or inhibit the complications such as cancer (Koche *et al.*, 2016). In the last few decades, phytochemicals have received much more attention because of their antioxidants and medicinal properties (Zhang *et al.*, 2015; Altemimi *et al.*, 2017; Forni *et al.*, 2019).

Litchi (*Litchi chinensis* Sonn.*)* is one of the most delicious and popular common fruits in Southeast Asia and fresh fruit are rich in Vitamin C. Only the flesh of the litchi fruitis consumable and the rest of the parts, especially seeds are discarded. However, litchi seeds are rich in protein, carbohydrates, vitamins, minerals, and seed extract have a role in the prevention of fatty liver and are widely used in relieve neuralgic pain (Wang *et al.*, 2011; Shukla *et al.*, 2013; Sonia *et al.*, 2017). The seed extract is also used to reduce or control inflammation, allergy, diabetic, hyperlipidemia, pyretic, obesity, cardiovascular problem, viral infection, and different type of cancer, etc. (Xiao *et al.*, 2004; Wang *et al.*, 2006; Ibrahim *et al.*, 2015). However, as far as we know, there is no research work done on phytochemicals and their role in litchi seeds cultivated in Bangladesh. So, litchi seed can open a new window in food safety and the field of functional food or nutraceuticals. This study aims to detect antioxidant potential in various extracts (aqueous and ethanol) of Bombay litchi variety seeds collected from Rajshahi, Bangladesh and to discover the contextual connections of phytochemicals as antioxidant activities.

Materials and Methods

Preparation of sample

Litchi fruits (Bombay variety) were collected from Shaheb Bazar, Rajshahi, Bangladesh in the last week of May, 2019. About one kg seeds were collected, washed, dried in sunlight, and finally ground to fine powdered to uniform particle size and stored in an airtight container for extraction and isolation of phytochemicals. All chemicals used in this study were of analytical grade.

Seed extract preparation

The seed powder (1 g/20 mL) was soaked in extracting media (water and 30% ethanol) at 4°C for 72 hours with occasional shaking. The samples were filtered with muslin cloth and finally with Whatman 42 filter paper and collected supernatant one. The precipitate one was (about 1 g/10 mL) soaked again in extracting media at 4ºC for one hour for extraction of rest of the part of phytochemicals; filtered and collected the supernatant. All the supernatant were collected and evaporated at 90-100ºC still dried (Zhang *et al.,* 2018; Chunli *et al*., 2015). The residues were collected and used for qualitative and quantitative investigation of phytochemicals.

Qualitative analysis of phytochemicals

The different phytochemicals were qualitatively studied by using the following methods (Eke *et al.,* 2014).

Alkaloids test

The alkaloids were identified by Meyer's reagent. The extract residues (0.1 g) were defatted with 5% ethyl ether for 15 min and followed for 20 min in 5 mL of 1 N HCl on steam. After centrifugation of the sample, one or two drops of Meyer's reagent were added and the result was recorded.

Tannins test

The presence of tannins was analyzed by adding a few drops of 5% $FeCl_3$ solution in a test sample (0.1 g of dried sample per 2 mL distilled water) and the result was noted.

Flavonoids test

The dried powder was dissolved in 10% ethanol (0.1 g/1 mL) and then 1 mL sample was mixed in 5 mL diluted ammonia and followed by a few drops of concentrated H_2SO_4 for qualitative analysis of flavonoids. The effect was recorded.

Saponins test

To analyze the saponins compound, add a drop of 1 N sodium bicarbonate solution in 2 mL (0.1 g of dried sample in 2 mL distilled water) solution then shaken vigorously, left for 3 minutes and finally the result was documented.

Terpenoids test

The terpenoids were qualitatively analyzed by the addition of 2 mg of dry powder in acetic anhydride solution in a test tube, cooled, and added 1 mL of conc. H_2SO_4. The outcome was noted.

Steroids test

For steroids analyses, 2 mL solution (0.1 g of dried powder in 2 mL distilled water) was added in chloroform (2 mL) and added 3-4 drop of conc. H_2SO_4, mixed well. The result was noted. The presence of resins was determined by dissolving 0.1 g of dried extract in acetone, the solution was poured in distilled water and the result was recorded.

Total phenolic content (TPC) estimation

The TPC was measured by FCR (Folin-Ciocalteu's Reagent) method using a spectrophotometer (Kaur and Kapoor, 2002). Dried powder (0.05 mg/mL in ethanol) was used in the determination of TPC. The reaction mixture was made by mixing 0.5 mL of sample with 2.5 mL diluted FCR (10 times diluted), and 2.5 mL of 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 mL ethanol, 2.5 mL of diluted FCR and 2.5 mL of 7.5% of $NaHCO_3$. After 25min, the absorbance was recorded at 760nm. All the experiment was done at least in triplicate and the mean value was documented. The calibration curve was prepared by the different concentrations of gallic acid (GA) (Fig. 1) as standard agent. The TPC was expressed in terms of GA equivalent.

Measurement of flavonoids

The flavonoids are expressed as flavones and flavonols. The total flavonoid contents (TFC) was calculated from the crude extract of litchi seeds by using a colorimetric described by Chang *et al.*, 2002. The dried powder was dissolved in ethanol and made up 0.05 mg/mL. One mL sample was mixed in 0.5 mL of 5% $NaNO_2$ and 0.5 mL of 10% $AlCl_3$ and incubated for 10 min. After incubation, 2 mL of 1 M NaOH was mixed. After 15 min, the absorbance was taken at 510 nm. The calibration curve was made by the different concentrations of Catechin as a standard substance (Fig. 2). TFC was calculated and expressed in µg/mL.

Determination of total antioxidant activity (TAA)

TAA was measured by the FRS method of DPPH (1,1-diphenyl-2-picrylhydrazyl); described by Prieto *et al.*, 1999 and Mensor *et al*., 2001 with a little modification by Re *et al.*, 1999 called $ABTS^+$ (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) method. In this method, 0.1 mM of DPPH in ethanol and the 1.0 g of dried litchi powder was mixed, centrifuged and the clear solution was used in this experiment. 5 mL of sample at different concentrations was taken in different test tubes and added 3 mL of 0.1 mM DPPH in each tube. Control was made without sample. The absorbance was recorded at 517 nm after 30 min and converted into the percentage of antioxidant inhibition activity of DPPH using the following equation:

$$\text{Inhibition (\%)} = (A_0\text{-}A_1)/A_0 \times 100$$

(Where, A_0 was the abs. of the control, A_1 was the Abs. of the sample or ascorbic acid)

Ascorbic acid was used as standard and TAA of sample that was compared to antioxidant activity of ascorbic acid. The results were also expressed as µg sample/mL. The IC_{50} value means 50% of the free radical was inhibited of DPPH by ascorbic acid or the sample.

The antioxidant activity of litchi seed was measured by $ABTS^+$ method. 7 mM ABTS and 2.45 mM potassium persulfate (1:1) was mixed and stored at dark room to oxidative stable form for 12-16 h to form free radicals and used as a stock solution. Three mL of stock solution was mixed in 1 mL of ascorbic acid or sample at different concentrations; stand for 6 min and the absorbance was recorded at 735nm. Ethanol and ascorbic acid was used blank and standard, respectively. Antioxidant activity was expressed as-

$$\text{Inhibition (\%)} = (A_0\text{-}A_1)/A_0\times 100$$

(Where, A_0 is the abs. of ethanol and A_1 is the abs. of sample or ascorbic acid)

The results were also expressed as µg sample/mL and IC_{50} means that the amount of ascorbic acid or sample to inhibit 50% free radicals activity that calculated by the calibration curve. All the experiment was done at least triplicate and average result was used for data analysis.

Statistical analysis

The results were presented as standard deviation as mean ± standard error (SE) of triplicate measurements.

Results and Discussion

Qualitative analysis of phytochemical

The qualitative analysis or screenings of phytochemicals of litchi seeds of alcoholic and aqueous extract are summarized in Table 1. The phytochemicals screening pointed out that most of the phytochemicals were present in the litchi seed extract in 30% ethanol. Most of the phytochemicals like alkaloids, flavonoids, terpenoids, steroids, tannins, and resins were extracted in ethanol whereas; terpenoids, steroids, and tannins were extracted in aqueous media. Only saponin was not present in ethanolic extract (Table 1). Therefore, it can be suggested that ethanol is a suitable extracting media for the extraction of phytochemicals from the litchi seed. A similar result was published by Eke *et al.*, 2014.

Table 1. Phytochemical evaluation of alcoholic and aqueous extract

S/N	Phytochemicals	Observation	Ethanol extract	Aqueous extract
1.	Alkaloids	A turbid ppt was observed	+ve	-ve
2.	Flavonoids	A brown ring was formed	+ve	-ve
3.	Terpenoids	Pink color was formed	+ve	+ve
4.	Steroids	Red color was observed	+ve	+ve
5.	Tannins	Blue-black ppt was formed	+ve	+ve
6.	Saponins	No change	-ve	-ve
7.	Resins	Turbid was formed	+ve	-ve

Quantitative analysis of phytochemical

In this work, quantitative phytochemicals content was determined from the litchi seed extract in 30% ethanol and water. The ethanol extract contained 6.18% phytochemicals in seed powder extract whereas; the water extract contained 5.84% (Table 2). The raw seeds contained 3.09% and 2.92% phytochemicals in ethanol and

water extract, respectively because 50% weight was decreased when fresh litchi seeds were dried into a powder. Alcohol was the suitable extracting media of phytochemicals from the litchi seed. The similar result was reported by Shukla *et al.*, 2013; Eke *et al.*, 2014; Iqbal *et al.*,2019; Rosales *et al.*, 2019. So, the rest of the studies were done by using 30% ethanol extract residues.

Table 2. Phytochemicals content of litchi seeds by different extracting media

Extracting media	Weight of seed (g)	Weight of powder (g)	Yield/Dried wt. (g)	Yield in dried (%)
Ethanol	20	10	0.618±0.026	6.18
Water	20	10	0.584±0.031	5.84

Total phenolic content

The phytochemical substances of litchi seeds were measured in terms of TPC and TFC. As, it has been accepted that these compounds are present in natural plant and plant products; serves as a potent antioxidant and stabilize or neutralize the free radicals which are responsible for oxidative damage. TPC was measured by using a calibration curve made by different concentration of gallic acid as standard substance (Fig. 1) and phenolic substance was measured from the litchi seed extract. The TPC was calculated as 19.86 mg/g of dried litchi seed and 9.98 mg/g of fresh litchi seed (Table 3).

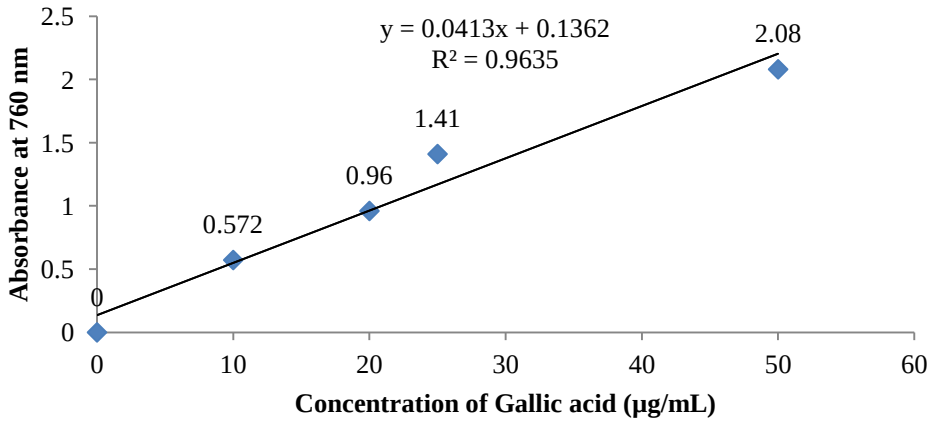

Fig. 1. Standard curve of Gallic acid

Total flavonoids content

The Flavonoids component was measured 20.6 mg/g of dried litchi seed powder and 10.3 mg/g of fresh litchi seed (Table 3). TFC content was measured using a calibration curve made by different concentration of Catechin as standard substance (Fig. 2).

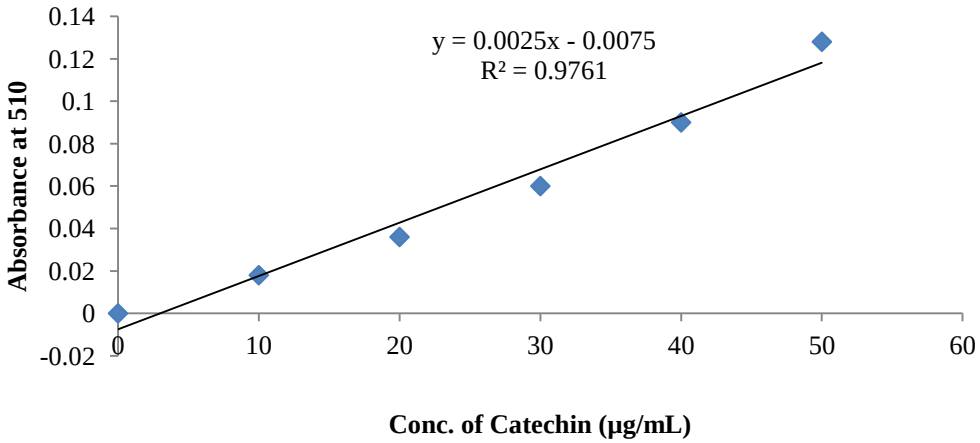

Fig. 2. Standard curve of Catechin

Table 3. Total phenolic and flavonoid content of litchi seed

Phytochemicals	Amount in dried powder (mg/g)	Amount in fresh litchi seed (mg/g)
TPC	19.86±1.90	9.98±0.98
TFC	20.60±2.17	10.32±1.04

Antioxidant activity

The antioxidant activities of different concentrations of seed extract and standard ascorbic acid was exhibited in a dose dependent manner of free radical scavenging assay. The litchi seed phytochemicals exhibited significant antioxidant activity and the activity of litchi seed phytochemicals were compared with standard ascorbic acid (Table 4). The result was shown in Table 5 that showed litchi seed phytochemicals exhibited almost same effectiveness to ascorbic acid. The IC_{50} value of litchi seed phytochemicals and ascorbic acid were also calculated and the value of litchi seed phytochemicals and ascorbic acid was 274.92 and 240.42 µg/mL, respectively (Table 6). The scavenging activity of litchi seed phytochemicals was increased with increasing concentration and the IC_{50} value of litchi seed phytochemicals and ascorbic acid were 31.44 and 29.33 µg/mL, respectively (Table 6).

Discussion

The phytochemicals investigation in this study revealed that alkaloids, flavonoids, terpenoids, steroids, tannins, and resins were existent in litchi seed (Table 1) which is supported by the previous studies cited by Eke *et al.*, 2014. The litchi seed is one of the richest sources of phytochemicals (6% in dried seed) and maximum phytochemicals

Table 4. Free radical of DPPH was inhibited by the antioxidant activity of litchi seed phytochemicals and ascorbic acid

Conc. of antioxidant (µg/mL)	Inhibition by phytochemicals (%)	Inhibition by ascorbic acid (%)
Blank	0	0
100	15	22
200	36	44
300	54	71
400	82	92
500	94	95

Table 5. Free radical of $ABTS^{+}$ was inhibited by antioxidant compound of litchi seed and ascorbic acid

Conc. of antioxidant (µg/mL)	Inhibition by phytochemicals (%)	Inhibition by ascorbic acid (%)
Blank	0	0
10	24	26
20	39	41
40	61	66
60	73	78
80	86	91

Table 6. The anti-oxidant capacity of litchi seed phytochemicals and ascorbic acid in terms of IC_{50}

Antioxidant assay method	IC_{50} of ascorbic acid (µg/mL)	IC_{50} of seed phytochemicals (µg/mL)
DPPH assay	240.42±2.70	274.92±2.40
ABTS assay	29.33±2.90	31.44±3.10

were extracted in ethanol (Table 2). The result is supported by Shukla *et al*., 2013 and Rosales *et al*., 2019. The TPC and TFC were determined at 1.98% and 2%, respectively from the dried litchi seed (Table 3). The results showed that litchi seeds are the richest sources of bioactive compounds such as TPC, TFC, etc. compare to the other sources of Iqbal *et al.,* 2019 and Rosales *et al.,* 2019 and related results were also described by Shukla *et al*., 2013. Phenolic compounds are broadly distributed in plant tissues, mainly contributing color and flavor of flowers, fruits or seeds.

The concentration of phenolic compounds may range from 0.5 to 5.0 g/100g dry weight of plant tissues (Swanson, 2003). The present results indicated that the litchi seeds

are the richest sources of phytochemicals. The phytochemicals contain many phenolic groups. The phenolic group/ring of phytochemicals is associated with antioxidant abilities. Not only do they exhibit antioxidant activity, but they are also in volved to reduce many diseases and play a fruitful role in human health (Minatel *et al.*, 2017). The free radical scavenging activity of phytochemicals is recognized as the capacity of neutralized free radicals, donating a proton, capturing electrons, or detoxification of heavy metal ions (Bendary *et al.*, 2013).

The antioxidant activity of litchi seed phytochemicals is summarized in Table 4-6. The IC_{50} value of litchi seed phytochemicals was 274 and 31µg/mL for DPPH and ABTS scavenging methods, respectively; however, for ascorbic acid that was 240 and 29 µg/mL, respectively. The results indicated that litchi seed phytochemicals showed strong antioxidant activity capacity in both antioxidant scavenging methods (Table 4-6). The results revealed that the phytochemicals of litchi seed act as a strong antioxidant identical to ascorbic acid (Table 6). Paliga *et al*., 2017 and Shukla *et al.*, 2013 reported the antioxidant activity of litchi seed phytochemicals higher than 78.36%. Ibrahim and Mohamed, (2015) reviewed the chemical constituents and pharmacological activities of litchi seed, listing the single constituents known over the past few decades. It is well evident that the litchi seed is one of the richest sources of phytochemicals, and these phytochemicals are used as strong antioxidants. Free radicals are unavoidably formed in life naturally and captured or remove hydrogen or proton, creating oxidative damage; as a result different types disorders degenerate. Three main steps initiation, propagation, and termination are mediated by free radicals in our body. The antioxidant substances fight against free radicals and protect our bodies from serious diseases. The main sources of these antioxidants are plants. Human beings consume antioxidants through diet and fight against free radicals. Several phytochemicals possessing polyphenolic structures have been advocated as nutraceuticals and used as food supplements for better healthcare in recent years (Halliwell, 1996). Flavonoids consist of many indispensable substances which have a great beneficial effect on health and are used as nutraceuticals. The components are also used for pharmaceutical, medicinal and cosmetic purposes (Halliwell, 1996). From our study, it is revealed that the litchi seed is one of the richest sources of phytochemicals that act as antioxidants and can play a great role against different diseases and disorders in the body.

Conclusion

L. chinensis is one of the most delicious and popular common fruits in various countries of world not only due to its juicy arils and nutritional benefits but also for pharmacological activities against various ailments. Only flesh of the litchi fruit is consumable and the rest of the parts, especially seeds are discarded. The phytochemical constituents present in seeds of *L. chinensis* illustrated that the seeds may be advocated as nutraceuticals as food supplements for better healthcare, or used both in a pharmaceutical

and dietary supplement for human being as well as animals. As a result, the wastage or discarded one can be used to economical one.

Conflicts of Interest

We have no conflicts of interest.

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